World Journal of Microbiology & Biotechnology **14**, 895–901

Use of randomly amplified polymorphic DNA analysis to differentiate isolates of *Vibrio parahaemolyticus* from cockles (*Anadara granosa*)

R. Son,* E.H. Nasreldin, H. Zaiton, L. Samuel, G. Rusul and F. Nimita

A total of 35 Kanagawa-negative strains of *Vibrio parahaemolyticus* isolated from cockles (*Anadara granosa*) were investigated by randomly amplified polymorphic DNA fingerprinting with three primers and their plasmid profiles. Eighteen strains carried small plasmid(s) of 2.4 to 7.3 kb that enabled the *V. parahaemolyticus* to be grouped into eight plasmid patterns. The three primers generated polymorphisms in all 35 strains of *V. parahaemolyticus* tested, producing bands ranging from 0.25 to 3.9 kb. The RAPD profiles revealed a high level of DNA sequence diversity within the *Vibrio parahaemolyticus* strains tested, and that cockles in the study area are populated by genetically polymorphic strains of *V. parahaemolyticus*.

Key words: *V. parahaemolyticus*, plasmid, RAPD, cockles (*Anadara granosa*).

Vibrio parahaemolyticus is a halophilic gram-negative bacterium which is widely distributed in estuarine and marine environments (Kaneko & Colwell 1973). The pathogenicity of this organism is believed to be associated with a lethal toxin (Honda *et al*. 1976; Sarkar *et al*. 1987), a vascular permeability factor (Honda *et al*. 1976), and thermostable direct haemolysin and related haemolysins (Nishibuchi *et al*. 1989; Honda *et al*. 1989; Taniguchi *et al*. 1990). Human infections with *V. parahaemolyticus* are usually linked to the consumption of raw or mishandled seafood or through a wound (Joseph *et al*. 1982; Johnson *et al*. 1984) and the organism is an important agent of human gastroenteritis. Despite the ubiquity of *V. parahaemolyticus* in marine and estuarine environments, and in shellfish, there is great variability in the incidence and distribution in different regions depending on the seasons, faecal pollution, sample type and experimental variables (Bartley & Slanetz 1971; Watkins & Cabelli 1985; DePaola *et al*. 1988; Depaola *et al*. 1990. Hence, due to the fact that most strains of environmental and seafood isolates are likely to be avirulent, it may prove difficult to correlate the presence of *V. parahaemolyticus* in shellfish with the development of disease in humans. Thus, the ability to characterize and determine relatedness among bacterial isolates involved is a prerequisite for epidemiological investigations. With the rapidity with which people and goods move within and between countries, there is a need for rapid and accurate typing techniques to assist public health authorities in the detection and tracking of related outbreak strains. Randomly amplified polymorphic DNA (RAPD) PCR is a genotyping analysis method which has increasingly been used to compare strains of numerous bacterial species because of the generic capabilities of the PCR system. In addition, the determination of plasmid profiles can aid in the differentiation of isolates in epidemiological investigation. The present study characterized 35 *V. parahaemolyticus* isolates from cockles (*Anadara granosa*) by RAPD-PCR and plasmid profiles.

R. Son and L. Samuel are at the Department of Biotechnology, Faculty of Food Science and Biotechnology, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia. E.H. Nasreldin, H. Zaiton, G. Rusul and F. Nimita Department of Food Science, Faculty of Food Science and Biotechnology, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia. *Corresponding author: Dr. Son Radu, Faculty of Food Science and Biotechnology, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia; Tel.: +6 03 9486101 ext. 3446; Fax: +6 03 9423552; E-mail: son@fsb.upm.edu.my.

Materials and Methods

Sample Collection, Isolation and Identification

The *V. parahaemolyticus* population in cockles (*Anadara granosa*) were investigated. A total of 100 samples of cockles were pur-